Pilot experience of an External Quality Assurance for Acylcarnitines in plasma/serum

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Background

ERNDIM (www.erndim.org) and CDC (www.cdc.gov) offer an external quality assurance (EQA) scheme for qualitative and quantitative acylcarnitines (ACC) in dried blood spots, respectively. However, ACC are usually determined in plasma/serum samples in some biochemical genetics laboratories for diagnosis and/or treatment monitoring of organic acidurias or fatty acid oxidation (FAO) defects.

A pilot interlaboratory comparison experience between eleven European laboratories was initiated in 2012.

The aim of this experience was to improve the analytical performance and interpretation of acylcarnitines in plasma/serum samples.

Samples

Serum/plasma samples from the same individual with a confirmed diagnosis were pooled as remains from diagnosis or treatment monitoring. Samples were kept frozen and were used after confirming the presence of marker acylcarnitines. Sixteen different samples (60µL) with a short case report were circulated at room temperature by regular mail from Madrid or Copenhagen. Samples were received in 2-7 days.

ACC analytical methods

Participants were asked to precise the method used to quantify diagnostic ACC, to give reference values, possible diagnosis and advice for further investigations before three weeks. All labs used a MS/MS equipment. Five of eleven labs analyzed the ACC as butylated derivatives and 6/11 underivatized; 7/11 used the precursor ion acquisition mode and 4/11 multiple reaction monitoring (MRM); and only 3/11 separated the short-chain isomers.

Results

Reference values and pathological concentrations of ACC varied among laboratories. Figure 1 shows pathological and reference values for C16 and C18:1, marker ACC for CPTII or CACT deficiency, as an example of the variation of concentrations between the participants.

The increases of C3; C5; C8; C10:1; C10; C16; C18:1; C16OH; C18:1OH, C5-C18 were correctly identified by all labs, allowing the diagnosis of Propionic Acidemia; Isovaleric Acidemia; MCAD and MADD by all participants (Table 1).

Despite a good identification of [C16OH; C18:1OH] and [C16; C18:1], markers for LCHAD/MTP defects and CPTII/CACT defects, respectively, some labs suggested only one disease.

However, the increases of dicarboxylic acylcarnitines (C3DC; C4DC, C5DC, C6DC) were not always identified. Diagnosis was not correct as exemplified in cases of malonic aciduria or HMG-CoA Lyase defect.

Misinterpretation occurred in those labs that did not derivatize, separate isomers or use MRM acquisition. However some of these labs suggested further analyses to achieve the diagnosis.

Figure 2 shows an example of separation of isomers in a IBDH deficiency and a combined defect of SCAD and IVA.

Table 2. External Quality Assurance for Acylcarnitines (ACC) in serum/plasma (pilot experience)

Year/number of participants	Disease*	Marker ACC	Identification	Correct Interpretation
2012/4	HMGL	C5OH; C5DC	3/4	3/4
	IVA	C5	4/4	4/4
	MCAD	C8; C10:1; C10	4/4	4/4
2012/10	MMA	C3; C4DC	6/10 2/10: C5OH/C4DC	8/10
	LCHAD	C16OH; C18:1OH	10/10	8/10: LCHAD/MTP 2/10: LCHAD
	GAI	C5DC	8/10	8/10
2013/8	VLCAD	C14:1	7/8	7/8
	MADD	C5-C18	8/8	8/8
	PA	C3	8/8	8/8
2013/8	CACT	C16; C18:1	8/8	6/8: CPTII/CACT 2/8: CPTII
	β-KT	C5:1; C5OH	7/8	8/8
	Malonic aciduria	C3DC	4/8 1/8: C3DC/C4OH	5/8
2014/9	HCS	C3; C5OH	4/9: C3 5/9: C5OH (only 3 as 3OHisovaleryl) 1/9: C4DC/C5OH	1/9: HCS 2/9: MCC 3/9: MCD 3/9: other
	IBDH	isoC4 or C4	6/9: C4 (only 2 as isoC4)	2/9: IBDH 2/9:IBDH or SCAD 1/9: SCAD 1/9: SCAD or MADD
	MCC	C5OH; C5:1	7/9 C5OH (only 3 as 3OH isovaleryl) 2/9: C4DC/C5OH 2/9: C5:1	8/9: MCC 1/9: HCS or Biotinidase
	SCAD+IVA	C4; C5	8/9: C4 (only 3 as butyryl) 9/9: C5 (only 3 as isovaleryl)	2/9: SCAD+IVA 2/9: IVA 5/9: MADD

*HMGL: Hydroxy-Methyl-GlutarylCoA Lyase; IVA: Isovaleric Acidemia; MCAD: Medium-Chain AcylCoA Dehydrogenase; MMA: Methylmalonic Aciduria; LCHAD: Long-Chain 3-OH AcylCoA Dehydrogenase; GAI: Glutaric Aciduria type I; VLCAD: Very-Long AcylCoA Dehydrogenase; MADD: Multiple AcylCoA Dehydrogenase; PA: Propionic Acidemia; CACT: Carnitine Acylcarnitine Translocase; β-KT: β-Ketothiolase; HCS: Holocarboxylase Synthethase; IBDH: IsobutyrylCoA Dehydrogenase; MCC: MethylcrotonylCoA Carboxylase; SCAD: Short-chain AcylCoA Dehydrogenase; CPT II: Carnitine Palmitoyl Transferase; MCD: Multiple Carboxylase Defect

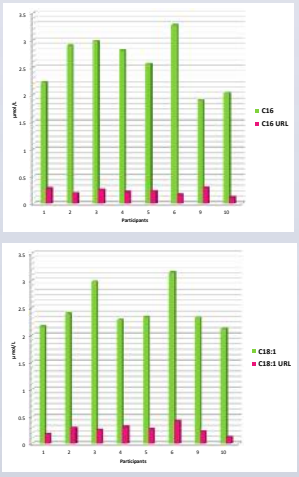

Figure 1. Pathological and reference values for C16 and C18:1, marker ACC for CPTII or CACT deficiency, in the different participant labs. URL: upper reference level

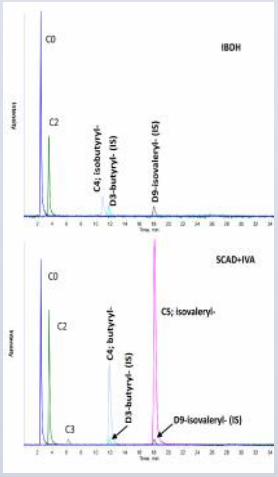

Figure 2. Separation of isomers in a IBDH deficiency and a combined defect of SCAD and IVA by HPLC/MS/MS. IS: internal standard.

Comments

This pilot experience highlights the importance of establishing reference ranges for ACC, the necessity of quantitative external quality assurance scheme for those commercially available ACC, and the importance of an interpretative EQA scheme for ACC in plasma samples.

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